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Response of soil organic and inorganic nutrients in alpine soils to a 16-year factorial snow and N-fertilization experiment, Colorado Front Range, USA

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Abstract

Alpine ecosystems are thought to be particularly sensitive to small environmental changes in climate and other parameters due to the plants and soil organisms being on the edge of environmental tolerances. Snow distribution is critical to microclimate in the alpine, affecting soil temperature, growing season duration, and nutrient cycling. Moreover anthropogenic nitrogen (N) deposition over the past half century has had a detrimental impact on temperate ecosystems, resulting in soil acidification and a reduction in plant biodiversity. Here we used a snowfence experiment combined with an N-fertilization experiment, at the NSF-funded Niwot Ridge (NWT) Long-Term Ecological Research (LTER) site (3528 m ASL), to increase our understanding of how changes in snow properties and N deposition may affect soil processes. The snowfence used in this manipulation resulted in a consistent pattern of snow accumulation, from deep snowpacks near the fence to a shallow snowpack away from the snowfence. As measured after 16 years of the experiment, the amount, timing, and duration of snow cover appears to affect soil properties. Under moderate snow cover and without N addition, the total soil organic carbon (TOC) and total nitrogen (TN) were significantly greater than either under deep or shallow snow. Nitrogen amendments in general worked in the opposite direction of snowpack controls on soil processes. The N addition caused a significant increase under the shallow snow treatments for TOC and TN, while there was a significant decrease of these properties under the moderate snow treatment. In the latter case the N addition didn't cause any significant effect on the inorganic N forms but was correlated with a decline of soil pH, and a consequent increase of exchangeable Al and a reduction of exchangeable base cations, which may have influenced soil microbial biomass found in this study. Our results demonstrate how long-term changes in snow properties and N deposition may interact in affecting alpine soil characteristics, with an important response of soil nutrients.

Keywords: Snow, soil, nitrogen, tundra

72 **Introduction**

73 Nutrient availability is an important factor for the functioning of an ecosystem, and is especially
74 critical in extreme environments. Alpine ecosystems are thought to be particularly sensitive to small
75 environmental changes in climate and other parameters due to the plants and soil organisms being
76 on the edge of environmental tolerances (Williams et al., 1998a, 2002). Snow redistribution by wind
77 action is critical to microclimate in the alpine, affecting soil temperature (Walker et al., 1993;
78 Williams et al., 2009), soil moisture (Taylor and Seastedt, 1994), decay rates (O’Lear and Seastedt,
79 1994), plant productivity (Walker et al., 1994), organic matter accumulation (Burns and Tonkin,
80 1982), species diversity (Litaor et al., 2008), soil genesis (Schaetzl, 1990; Holtmeier and Broll,
81 1992) and fluxes of trace gases such as CO₂, N₂O, and CH₄ (Brooks et al., 1997; West et al., 1999,
82 Filippa et al., 2009; Liptzin et al., 2009). It also influences microbial processes, which control gross
83 N mineralization and N immobilization among plant communities (Fisk et al., 1998, Litaor et al.,
84 2002; Edwards et al., 2007).

85
86 Most studies of environmental change in the alpine have focused on two key features: precipitation
87 and atmospheric deposition of dissolved inorganic nitrogen in wetfall ($\text{DIN} = \text{NH}_4^+ + \text{NO}_3^-$)
88 (Williams et al., 1998b). In areas with previously stable snow conditions, climate change may cause
89 a reduction in the depth, duration, and stability of the snow cover (Williams et al., 1998a). Milder
90 winters with thinner or less permanent snow cover are likely to affect the occurrence, depth, and
91 duration of soil frost and are associated with midwinter snowmelts (Austnes et al., 2008).
92 Nevertheless, even in a warming climate, some areas may experience deeper snowpacks and longer
93 snow duration and other areas may have lower snow cover and lower snow duration (Billings, 1968;
94 Williams et al., 2009). Precipitation, much of which occurs in the form of snow, has significantly
95 increased at the Niwot Ridge in the Colorado Front Range since measurements began in the 1950s
96 (Williams et al., 1996). Moreover at the mesotopographic scale there are large differences in snow
97 accumulation driven by the interaction of the snowfall with topography and wind (Figure 1).
98 Changes in wind speed could affect the historical patterns of snow distribution. In particular several
99 regional studies looking at the United States, Australia, China and parts of Europe have shown
100 decreasing wind speeds just above the planet's surface. Climate change, afforestation and urban
101 development had been suggested as possible causes (Vautard et al., 2010). Any changes in the type,
102 timing and total amounts of winter deposition have been shown to potentially feedback on carbon
103 (C) and nitrogen (N) dynamics (Liptzin et al., 2009, Monson et al., 2006; Brooks and Williams,
104 1999). Atmospheric deposition of DIN has increased three to four-fold at Niwot Ridge over the past
105 decades (Williams and Tonnessen, 2000) (Figure 2). Measures to reduce nitrogen emission have

106 begun to take effect, and deposition has a downward trend at least in some areas (Kelly et al., 2002).
107 But the deposition is still high; for instance the average deposition in Western Europe is
108 approximately 18 kg ha⁻¹ N, whereas the estimated background deposition is approximately 3 kg ha
109 ⁻¹ N (Galloway et al., 1984).

110 Anthropogenic DIN deposition over the past half century has had a detrimental impact on temperate
111 ecosystems in Europe and North America, resulting in soil acidification and a reduction in plant
112 biodiversity. During the acidification process, soils release base cations, such as calcium and
113 magnesium, neutralizing the increase in acidity. Once these base cations have been depleted,
114 aluminium is released from the soils, and can potentially reach toxic levels (Bowman et al., 2008).
115

116 Present levels of atmospheric deposition at Niwot Ridge and the Green Lakes Valley in the
117 Colorado Front Range are sufficient to cause N-saturation of surface waters (Williams et al., 1996;
118 Williams and Tonnessen, 2000; Elser et al. 2009). Experimental additions of N to alpine tundra
119 have caused changes in individual species abundance, increases in the soil solution concentrations
120 and leaching of nitrate, and increased net nitrification (Bowman et al., 2006; Bobbink et al., 2010).
121 The reduction in species richness due to experimental N addition occurred across all of the tundra
122 communities studied on Niwot Ridge (Seastedt and Vaccaro, 2001). Moreover, the fertilized plots
123 exhibited higher nutrient concentrations in both above- and below-ground plant material compared
124 to controls (Litaor et al., 2008). It has been hypothesized that these changes in plant characteristics
125 may best be explained by changes in soil chemistry that resulted directly or indirectly from the N
126 additions (Seastedt and Vaccaro, 2001).
127

128 Snow amounts have decreased in some areas of the western USA (Monson et al., 2006), while in
129 other areas an increase of snow deposition has been found, particularly in the Arctic (Sturm et al.,
130 2005). There, increases in snow depth and duration were driven in part by increases in air
131 temperature and shrub growth. At Niwot Ridge there is also an expansion of shrubs, which may be
132 due to an increase of snow deposition (Williams et al., 1996). Several researchers have tried to
133 simulate the effect of changes in the seasonal snow-cover on soil properties. Experimental snow-
134 removal, as a simulation of a lack of snowcover, has been carried out world-wide in the last decade
135 (Groffman et al., 2001; Decker et al., 2003; Freppaz et al., 2008), while other researchers have used
136 snowfences to experimentally manipulate snow accumulation (Williams et al., 1998; Nobrega and
137 Grogan, 2007), or grooming to change its density (Rixen et al., 2008). A decrease in winter
138 precipitation may result in shorter winter seasons, in more pronounced and more frequent
139 freeze/thaw cycles, and in more days with the soil temperature well below 0°C during winter. The

140 experiments sometimes indicate as a consequence of these phenomena a faster mineralization of N
141 (Panikov and Dedysh, 2000; Grogan et al., 2004; Freppaz et al., 2007), higher N₂O emissions
142 related to freeze thaw cycles (Sharma et al., 2006), and a reduction of respiration rates (Mariko et
143 al., 1994; Melloh and Crill, 1996; Brooks et al., 1997; Welker et al., 2000; Nobrega and Grogan,
144 2007). At the same time, a lower mineralization of N (Walker et al., 1999; Schimel et al., 2004),
145 lower N₂O emissions (Goldberg et al., 2008), an increase in respiration rates (Nielsen et al., 2001;
146 Goldberg et al., 2008), were also indicated as the consequence of comparable experimental
147 simulations, making it considerably difficult to derive any general conclusion (Henry, 2007). In
148 particular it's still uncertain if changes in snow cover may cause the same changes in soil properties
149 as increasing N deposition. Brooks and Williams (1999) had proposed that increasing snow depth
150 and duration result in increasing mineralization up to a maximum snow depth. Moreover it's still
151 unknown what the potential interaction may be between changes in snow depth and duration and N
152 deposition, with respect to soil properties such as N availability and soil acidification.

153
154 Here we used a long-term snowfence experiment, combined with a N-fertilization experiment, at
155 the Niwot Ridge Long-Term Ecological Research (NWT LTER) site, to increase our understanding
156 of how long-term changes in snow properties and N deposition may affect soil processes. The large
157 snowfence used in this experimental manipulation resulted in a consistent pattern of snow
158 accumulation, from early-accumulation and deep snowpacks near the fence to late accumulating
159 and a shallow snowpack away from the snowfence. The snowfence allowed us to investigate three
160 different pedoenvironments: a) a deep snow-wet meadow close to the snowfence, b) a shallow
161 snow-dry meadow some distance from the snowfence and c) a moderate snow-moist meadow in
162 between (Figure 1, 3). The snowfence was erected in 1993 and our soil measurements were made in
163 2009, providing a 16-year time frame for soil processes to differ. The main goals of this research
164 were to 1) characterize the soil properties under different snow cover conditions and 2) to examine
165 the reaction of the same parameters to N addition under different snow cover depths and duration.

166

167 **Material and Methods**

168 *The study site*

169 The influence of changes in snowpack timing, duration, and depth on an alpine ecosystem was part
170 of an ongoing study at Niwot Ridge, Colorado. The Niwot Ridge LTER site (3528 m ASL) is
171 designed to incorporate continuing studies over many years to assess the effects of changes in snow
172 pack properties on alpine ecology and biogeochemical cycles (Bowman and Seastedt, 2001). The
173 climate is continental, with a mean annual air temperature (MAAT) equal to -3.8°C and the mean

174 annual precipitation equal to 1006 mm (Williams et al., 1996). Daily mean air temperatures during
175 the winter are often below -20°C (Williams et al., 1998). Snowfall contributes about 80% to the
176 annual precipitation (Caine, 1996). Average wind speed is $10\text{--}13\text{ m s}^{-1}$ during the winter, and
177 westerly winds prevail at all seasons (Blanken et al., 2009). Due to redistribution of snow, soil
178 moisture is locally much higher (or much lower) than expected given the recorded amount of
179 precipitation (Litaor et al., 2008). Niwot Ridge is a gently undulating interfluvium that did not
180 experience Pleistocene Glaciation and therefore this area has more extensive soil development than
181 many other Colorado Alpine areas (Burns, 1980).

182

183 Prior to the construction of the snowfence, the area was an ecotone separating moist meadow and
184 dry meadow (Walker et al., 2001), underlain by Pergelic Cryobromel soils (Burns, 1980). The depth
185 of the A horizon varied from about 0.2 to 0.4 m, overlying unconsolidated granite parent material
186 (Burns, 1980). Soil pH ranged between 5.3 and 5.4 (Seastedt and Vaccaro, 2001), soil total organic
187 carbon (TOC) and total nitrogen (TN) (10-cm depth) ranged respectively between $130\text{--}200\text{ g kg}^{-1}$
188 and $9\text{--}15\text{ g kg}^{-1}$. The original vegetation cover was dominated by the sedge, *Kobresia*
189 *myosuroides*, with patches of the forb *Acomastylis* (= *Geum*) *rossii* (Walker et al., 1993).

190

191 *The snowfence*

192 The saddle of NWT is the site of a long-term snowfence study designed to assess the effects of
193 potential climate change on alpine ecology and biogeochemical cycles (Williams et al., 1998a). In
194 October 1993, a 2.6-m tall \times 60-m long snowfence of a composite Centaur® polymer wire rail with
195 a density of 50%, was installed in the saddle's dry alpine meadow perpendicular to the prevailing
196 westerly winds. The presence of the snowfence creates a gradient of snow depth and, consequently,
197 of snow onset, duration, and date of meltout with distance from the snowfence. Here, we
198 characterized the snow gradient into three snow depth sections (Figure 3): deep snow cover (D),
199 moderate snow cover (M), and shallow snow cover (S).

200

201 During the first winter of the experiment, the deep snow cover section increased 100 to 200% in
202 depth, and snow duration increased an average of 90 days compared to the pre-fence measurements
203 (Williams et al., 1998a). The increase in snow depth, almost constant through the years, because of
204 the aerodynamics of the snowfence, and snow duration resulted in underlying soil temperatures in
205 January increasing from about -15°C before installation of the snowfence to -5°C after installation
206 of the snowfence (Brooks et al., 1997). Because the wire rail of the fence can create localized

207 summer wind regime changes affecting the microclimate behind the fence, the wire rail is removed
208 each June for the non-snow season and re-installed each subsequent October.

209

210 *N addition plots*

211 Eight plots were placed in each snow depth sector in 1993. Each plot was 2 m × 2 m; specific
212 treatments of control (deep snow, control (DC), moderate snow, control (MC), and shallow snow,
213 control (SC)) and N additions (deep snow, nitrogen (DN), moderate snow, nitrogen (MN) and
214 shallow snow, nitrogen (SN)) were randomly selected. Each snow depth sector therefore contained
215 a two-factor factorial experiment for nutrient additions with four replicates per treatment. In the
216 summers of 1993, 1994, and 1995, 20 g m⁻² N was added as ammonium nitrate (NH₄NO₃ was 25%
217 of added N) and ammonium sulfate ((NH₄)₂SO₄ was 75% of added N). Beginning in 1996, plots
218 were fertilized using 10 g m⁻² N as (NH₄)₂SO₄. The logic for using ammonium as the dominant
219 form of inorganic N was to minimize leaching of nitrate. Plots were subsequently fertilized at 1996
220 rates for 1997, and 1998, and thereafter at these rates every other year. The plots were last fertilized
221 before measurements were made in 2008.

222

223 *Soil sampling and analysis*

224 The field campaign for this study was conducted in 2009. Soil samples were collected in 24 plots
225 using a standard soil corer during the snow free season (September 2009) on the leeward side of the
226 fence corresponding to deep, moderate and shallow snow pack depths. The soil colour was
227 determined in the laboratory after drying (dry) by the Munsell Soil Colour Charts. In the laboratory,
228 samples were dried and passed through a 2-mm sieve in order to separate the fine earth fraction
229 from the rocks (> 2mm). Soil moisture content was determined gravimetrically after drying at
230 105°C. Topsoil (A horizon) and subsoil (AB horizon) were analyzed for TOC and TN using a
231 LECO CHN-1000 CHN Analyzer, pH in water and exchangeable cations (Ca, Mg, Al).
232 Additionally, topsoil samples were processed for NH₄⁺ and NO₃⁻ pools, within 12 hours of returning
233 from the field. Fresh soils were sieved and homogenized using a 2-mm sieve. Subsamples of this
234 soil were extracted with K₂SO₄ 0.5M (1:5, weight: volume) by shaking at 250 rpm for 60 min and
235 allowing to sit at room temperature for 18 hours. These extracts were filtered through pre-rinsed
236 (300 mL distilled water) Whatman #1 filter paper, and aliquots were analyzed on the Lachat
237 autoanalyzer. NH₄⁺ was determined colorimetrically on a Lachat flow injection analyzer using a
238 phenolate reaction enhanced by nitroprusside. NO₃⁻ was analyzed using a sulphanilamide reaction
239 following reduction to nitrite on a cadmium column. To measure microbial biomass C (C_{mic}) and N

(N_{mic}), a set of samples was fumigated overnight with chloroform and extracted with K₂SO₄ 0.5 M in parallel with a set of unfumigated samples (Brookes et al., 1985).

For dissolved organic compounds, subsamples (10 g fresh weight) were shaken with 100mL 0.5M K₂SO₄ for 1 h and the suspension filtered at 0.45 µm under suction. Total dissolved N (TDN) in the extracts was measured as NH₄⁺ after oxidation of aliquots of extracts with alkaline persulfate.

We compare some of these results to soil cores that were collected before construction of the snowfence on the Niwot Ridge Saddle in the autumn of 1993. These 75 samples were analyzed for organic matter content, TOC, and TN using the same methods as above. These 1993 samples are representative of the baseline (pre-snowfence) soil conditions; raw values are available at <http://culter.colorado.edu/exec/extracttoolA?soilorgm.ts>.

The effects of snow and N addition on soil properties in the topsoil and subsoil were tested with a two-factor factorial ANOVA with Tukey Post Hoc Test. Analysis included Spearman's rho non parametric correlation analysis. Data analysis was performed using the SPSS statistical software. Graphs have been produced using R statistical software (R Development Core Team, 2010).

Results

Soil characteristics

The organic matter content was always higher in the topsoil than in the subsoil (p=0.001), with values ranging from 8.1% in the subsoil of SC to 23% in the topsoils of MC and SN (Table 1). The predominant soil colour was very dark brown in the topsoil, while in the subsoil it was more variable (e.g. dull yellowish brown in DC). The C/N ratio ranged between 12.9 in DC and MN topsoils and 14.5 in DC subsoil. Gravimetric water content at the sampling time (September 2009) ranged from 10.3% (subsoil SC) to 36.7% (topsoil MC), and was marginally higher in the topsoil (p=0.06).

In the snow manipulation plots without N addition (control plots), the TOC and TN concentration in the topsoil was significantly higher under the moderate snow cover (Figure 4) than under the deep and shallow snow covers (p<0.05). In the subsoil the TOC concentration was higher under the moderate snow cover, intermediate under the deep snow cover and the lowest under the shallow snow cover (Figure 5). In the deep and shallow control plots the TOC and TN content was significantly lower than the values recorded in 1993, before the snowfence setup, while a slight increase was recorded under the moderate snow cover plots (Figure 6 and 7). In the N addition plots the topsoil TOC and TN content was significantly higher under the shallow snow cover, while no

274 significant difference was found between the deep and moderate snow cover (Figure 4). The N
275 addition plots exhibited reduced amounts of TOC and TN both in the topsoil and subsoil under the
276 moderate snow cover, while an opposite trend was observed under the shallow snow cover. No
277 significant effect was found under the deep snow cover (Table 2). In the N addition plots the TOC
278 and TN content were significantly lower than the values recorded in 1993 in the deep and moderate
279 snow cover plots (Figure 6 and 7).

280

281 **Soil pH and Al**

282 The pH of the shallow control plot in the topsoil was about 5.6, significantly higher ($p=0.041$)
283 compared to that measured under the deep and moderate snow covers (Figure 8). In the subsoil the
284 pH under all controls was similar to that of the shallow control plot in the topsoil, near 5.6 (Figure
285 8). In the control plots, both in the topsoil and subsoil, a significant and negative correlation was
286 found between the pH and the TOC concentration ($r = -0.56$; $p=0.02$). The N addition plots
287 exhibited lower values of pH for the moderate and shallow snow covers and for both topsoil
288 ($p=0.001$) and subsoil ($p=0.001$) (Figure 8). The change in pH was most striking for the moderate
289 snow cover, which was about 4.4 for the topsoil and 4.7 in the subsoil. Using all results, a
290 significant and negative correlation was found between the pH and the DOC concentration ($r = -$
291 0.686 ; $p=0.007$).

292

293 In the control plots, the exchangeable Al concentration was less than $0.2 \text{ cmol}_c \text{ kg}^{-1}$, with no
294 significant differences among the different snow treatments, both in the topsoil and subsoil (Figure
295 9). Conversely, the N addition plots had increased values of exchangeable Al, particularly
296 evident under the moderate snow cover (Figure 9), with values greater than $3.3 \text{ cmol}_c \text{ kg}^{-1}$ for both
297 the topsoil and the subsoil. Considering both the control and N addition plots, a significant
298 correlation was found between pH and exchangeable Al in the topsoil ($r=-0.903$, $p<0.01$) and
299 subsoil ($r=-0.933$, $p<0.01$).

300

301 **Soil exchangeable base cations**

302 The exchangeable base cations in the control plots ranged widely, from about 10 to more than 15
303 $\text{cmol}_c \text{ kg}^{-1}$ for Ca and 1.2 to $2.4 \text{ cmol}_c \text{ kg}^{-1}$ for Mg (Figures 10 and 11). The concentration of Ca and
304 Mg in the control plots was not significantly different among snow treatments. The exchangeable
305 Ca and Mg were higher in the upper horizon than in the subsoil ($p<0.001$). The N addition caused a
306 significant decrease of exchangeable Ca and Mg (Figures 10 and 11) in the topsoil and subsoil
307 under moderate snow cover, while no significant effect was found under the deep and shallow snow

covers, though in the latter case a small increase was measured. Moreover the N addition caused a significant reduction of exchangeable Mg in the subsoil under the deep snow treatment.

Topsoil N and C forms

In the control plots the NH_4^+ and NO_3^- concentrations were significantly greater under deep and moderate snow cover than under the shallow snow cover (Table 3). The K_2SO_4 -extractable DOC and DON concentrations in the control plots were significantly greater under the moderate snow cover (Table 3, $p = 0.001$). Similarly, N_{micr} and C_{micr} concentrations were all significantly greater under the moderate snow cover (Table 3).

The N addition plots exhibited increased NH_4^+ and NO_3^- concentrations only under the shallow snow cover and of NH_4^+ under the deep snow cover (Table 3). The N plots showed increased DOC concentrations under the shallow snow cover (Table 3). In contrast to the control plots, the N addition plots exhibited decreased N_{micr} and C_{micr} concentrations under the moderate snow cover, while no significant effect was recorded under the deep and shallow snow covers (Table 3). The $\text{C}_{\text{micr}}/\text{N}_{\text{micr}}$ ratio ranged between 8.01 in DC and 9.17 in MN (Table 3), and significantly decreased in the N addition plots under the shallow snow cover ($p < 0.05$).

Results from the general linear model (2-way ANOVA) support the observation that the snow manipulation resulted in a significant effect on almost all C and N variables, excluding NH_4^+ ($p = 0.577$) and NO_3^- ($p = 0.667$). In the latter cases a significant interaction between the snow depth and N addition was found ($p < 0.001$; $p < 0.05$). Among the tested variables, only N_{micr} ($p = 0.067$) and C_{micr} ($p = 0.061$) did not show a relationship with N addition (Table 4 and 5), while a significant interaction between snow depth and N addition was found ($p < 0.001$).

Discussion

The soils at Niwot Ridge appear similar to tundra soils in Europe and at high latitudes, revealing a good incorporation of organic matter in these organo-mineral horizons. The TOC content ranged from 47 to 134 g kg^{-1} , slightly higher than values reported for example in the A horizons of tundra soils in the Italian Alps (Freppaz et al., 2010). These values are comparable to the values recorded in mountain grassland soils of the Pyrenees (Garcia-Pausas et al., 2007), at elevations ranging from 1845 m ASL to 2900 m ASL, and to the values reported for Arctic and Alpine tundra by McGuire et al. (1997), without considering the most recalcitrant fractions.

342 Over the 16 years of the snowfence experiment, the amount, timing, and duration of snow cover
343 appear to affect soil TOC and TN content. The soil under the moderate snow cover, without N
344 addition, revealed a significantly greater TOC and TN content than either under deep or shallow
345 snow (Figure 4 and 5). Brooks and Williams (1999) developed a conceptual model to understand
346 how the seasonal snowpack may control under-snow microbial activity and the release/retention of
347 nitrate. That model may help explain the differences in soil nutrients that we report for differences
348 in snow accumulation. Our moderate snow cover corresponds to zone III of Brooks and Williams
349 (1999), where the snow cover develops early in the season and soils typically do not experience
350 severe freeze/thaw events. Here free water is available throughout the winter and heterotrophic
351 activity continues through the winter and N retention is relatively high. By the comparison with the
352 TOC and TN content in 1993, before the snowfence setup, under the shallow (S) and deep (D) snow
353 cover there was a significant reduction of both TOC and TN content. At sites with a very short
354 duration snow cover (zone I in Brooks and Williams (1999) and sites S in our study) the soil
355 remains frozen through much of the winter, there is very little free water available, and over-winter
356 heterotrophic activity is very low. Consequently, there is a very weak N sink and N leachate is high.
357 Here the vegetation is sparse and consequently we expect low production of OM. In zone IV of
358 Brooks and Williams (1999) (D in our study) snow cover is present for much of the year,
359 occasionally never melting in large snow years. Microbial activity under snow in zone IV is reduced
360 because there is very little primary production during the growing season to provide carbon
361 substrate. In these areas there is a weak N sink during snowmelt and NO_3^- stored in the seasonal
362 snowpack contributes directly to snow melt runoff. These limiting factors could result in a lower
363 concentration of TN in the upper horizons, in comparison to the moderate snow cover area, where
364 the N retention is relatively high.

365 Moderate snow depths may lead to an ideal combination of moister and warmer soil conditions that
366 result in substantially increased C accumulation relative to deeper or shallower snow depths.
367 Williams et al. (1998a) have shown that there is an increased rate of litter decomposition (up to
368 50%) under deeper and earlier snowpacks at the Saddle site on Niwot Ridge that can result in
369 increased rates of C and N mineralization with earlier and deeper snow. Our results and those of
370 Williams et al. (1998a) are consistent with previous litter decomposition experiments by O'Lear and
371 Seastedt (1994) on Niwot Ridge that have shown that alpine litter does exhibit significant decay
372 under snow. The snowpack enhances moisture content and increases winter soil surface
373 temperatures, stimulating surface decomposition. The reduction of soil organic matter under the
374 deepest snowpacks is consistent with Webber et al. (1976), who speculated that excessive snow
375 cover is expected to ultimately reduce decomposition. Thus, these results are consistent with Zone

III of Brooks and Williams (1998), with the addition that moderate snow depths result in an increase in microbial biomass, higher rates of mineralization and decomposition, which in turns results in higher soil C, N and organic matter content.

Soil conditions under alpine snow packs can be very favourable to microbial growth (Brooks et al., 1998; Lipson et al., 1999) and the highest populations of microbes on an annual basis occur during the winter in alpine tundra soils (Lipson et al., 1999, Schadt et al., 2003). Our results show that microbial biomass C and N concentrations were significantly greater under the moderate snow depths when compared to deep and shallow control snowpacks. Microbial biomass contained a relatively constant proportion of TOC (0.8-1.2%). These percentages are lower than what reported for example by Cheng and Virginia (1993), across seven Alaskan tundra sites (2.5-2.7%). The same authors reported that the N incorporated in the soil microorganisms was about 7% of the total soil N. In our study the values are lower, ranging from 1.3 to 1.8%, with percentages slightly higher in the control plots than in the N addition plots.

The pH of the shallow control plot in the topsoil of about 5.6 is on the lower edge of soil pH values reported by Burns (1980) for Niwot Ridge. Here, the large additions of inorganic N as well as the addition of sulphate in the fertilizer amendments beginning in 1996 likely created conditions for soil acidification. The fact that pH declined in fertilizer treatments should not be surprising. However, the fact that the various treatments exhibited variable responses in soil pH show that the snow amounts and subsequent community response altered rates of soil acidification. The significantly lower pH values under the moderate snow depths reflect increased mineralization rates related to the snow conditions. Higher rates of mineralization under the deeper snow may move the system towards net nitrification, which would result in lower soil pH. Higher mineralization rates and lower soil pH is consistent with the increased amounts of soil microbial biomass that we report under the moderate snow depths. Exchangeable aluminium values in the control plots show little variation and are consistent with the relatively high soil pH values (Bowman et al., 2008).

The elevated calcium and magnesium values in the topsoil of the moderate and deep snowpacks relative to the shallow snowpack may reflect aeolian deposition. At the NWT LTER site, we know that aeolian deposition is an important source of base cations (Litaor, 1987; Rhoades et al., 2010). The majority of annual dust deposition occurs as dust on snow events, with much less dust deposition during the summer. Dust is entrained in snow, and dust deposition in this region during the period has been large (Corey et al., 2009), maybe buffering the higher leaching due to the

greater snow depth. Regions with large annual inputs of loess, such as Prudhoe Bay, Alaska, have nonacidic snowbeds and support relatively rich plant communities (Walker et al., 2001b). For example, the amount of dust trapped over the winter and spring of 1997-1998 in the seasonal snowpack at the NWT LTER site was 43 kg ha^{-1} , compared to a summer value of 7.7 kg ha^{-1} (Ley et al., 2004). The Ca^{2+} and Mg^{2+} concentrations of a dust event snowfall in the Southern Rocky Mountains in February 2006 were respectively 35 and 9-fold higher than previous snow (Rhoades et al., 2010). Most likely, the deeper the snowpack, the more aeolian deposition of dust. Thus, there may be higher amounts of calcium and magnesium loading from dryfall under the moderate and deep snowpacks compared to the shallow snowpack. Aeolian deposition as an important source of exchangeable calcium and magnesium is also consistent with higher values in the topsoil compared to the subsoil. The felsic crystalline rocks that underlie the soils on Niwot Ridge (Williams et al., 2006) weather slowly and release only small amounts of calcium and magnesium relative to aeolian inputs.

Nitrogen amendments in general worked in the opposite direction of snowpack controls on soil processes. For both the topsoil and the subsoil, the N addition caused a significant increase under the shallow snow treatments for soil organic matter, and TN, while there was a significant decrease of these values under the moderate snow treatment (Table 2). Similarly, while there was a significant decrease in microbial biomass C and N under the moderate snowpack with N fertilization, the N-amendments resulted in either no change or an increase in these variables for deep and shallow snowpacks.

Brooks et al. (1996, 1997, 1998) have shown that carbon and nitrogen mineralization along with microbial biomass are inhibited under shallow snowpacks when compared to deeper snowpacks because consistent snow cover insulated the soil surface from extreme air temperatures and allowed heterotrophic activity to continue through much of the winter, while under shallow snowpacks the soil remained frozen and production did not begin until snowmelt. However, Seastedt and Vaccaro (2001) showed that N-fertilization at the shallow snow cover sites enhanced net primary production. Moreover, we show that the increase of inorganic N forms due to the N addition in shallow snow sites was significant. These results are consistent with Schmidt et al. (2004) in dry meadow tundra soils at Niwot Ridge who found a significant increase both of ammonium and nitrate after N fertilization. Under the shallow snowpack, the pH of 5.6 for topsoil and 5.4 for subsoil for controls were both reduced to a pH of 5.0 after N-fertilization. The net production of hydrogen ions is consistent with N-fertilization enhancing mineralization activities under shallow snowpacks. Thus,

444 for shallow snowpacks (and possibly to some extent under deep snowpacks), N-fertilization may
445 compensate for the lack of inorganic N production from under-snow mineralization, resulting in an
446 increase in net primary productivity, soil organic matter, and TN, as well as inorganic N pools.

447
448 An intriguing question is why the levels of soil organic matter, TN, and microbial biomass were
449 lower in soils from fertilized plots under moderate snowpacks than in soil from control plots. In the
450 moderate snow cover areas, nitrogen fertilization has allowed for a grass (*Deschampsia* sp) to
451 replace a forb (*Geum* sp; Bowman et al., 1995). This grass produces litter with a higher
452 decomposition rate (Stelzer and Bowman, 1998), which may have resulted in an overall soil organic
453 matter decline.

454
455 Microbial biomass and activity have been shown to be significantly suppressed by mid- to long-
456 term (> 1 year) additions of nitrogen in a number of different studies (Fisk and Fahey, 2001;
457 Prescott et al., 1992; Söderström et al., 1983). Nitrogen fertilization not only increases levels of
458 nitrogen in the soil but also can decrease soil pH, even in already acidic soils (Aerts and de Caluwe,
459 1999; Fisk and Schmidt, 1996). This was the case in the present study because the pH under the
460 moderate snow cover averaged 4.3 in plots receiving N compared to 5.3 for control plots. It is not
461 clear if this sharp pH change alone could be the cause of the reduced microbial biomass recorded in
462 this study. The effect of fertilization could be higher in the summer, when soils were sampled, than
463 in the winter and this is consistent with recent findings that there is a profound microbial
464 community shift from summer to winter in tundra soils (Lipson et al., 2002). The C:N ratio of the
465 chloroform-labile microbial biomass ranged from 8 under deep snow cover without N addition and
466 9.1 under shallow snow cover without N addition and under moderate snow cover with N addition.
467 A significant reduction of the microbial C/N ratio due to the N addition was recorded only under the
468 shallow snow cover, as reported also by Schimdt et al. (2004), who in alpine tundra soils reported a
469 C/N ratio of 8.3 in the control plots and of 5.0 in the fertilized plots, indicating an increased
470 capacity to immobilize N in the fertilized plots.

471
472 The reduction of exchangeable base cations due to N addition was more significant under the
473 moderate snow cover, lowering their availability as nutrients and decreasing their potential
474 contribution to soil buffering capacity. Consequently a lower plant biomass production could be
475 expected, with a decrease of organic matter input into the soil which could contribute to explain the
476 reduced soil organic matter content recorded under the moderate snow cover after N addition. In the
477 same experimental plots, the N addition strongly reduced the plant species richness (Seastedt and

478 Vaccaro, 2001). Bowman et al. (2008) in the Western Tatra Mountains of Slovakia reported how
479 the above-ground plant biomass decreased with increasing inputs of inorganic N in the study plots.
480 Multiple decades of elevated anthropogenic N deposition may have alleviated any pre-existing N
481 limitation of production, and simultaneously exacerbated plant P limitation through higher P
482 occlusion with increasing soil acidification, as reported by Bowman et al., (2008).
483 From these results it appeared how any changes in the snow distribution pattern could strongly
484 influence the soil properties, altering the mineralization processes, and consequently with an
485 important response of soil nutrients. The N addition in general worked in the opposite direction of
486 snowpack controls on soil processes, resulting in an interaction between these factors, with
487 significant effect on high elevation seasonally snow covered pedoenvironments.

488

489 **Conclusions**

490 A greater content of organic matter and microbial biomass occurred under the moderate snow cover
491 than under the other snow treatments. Under the moderate snow cover N additions caused a sharp
492 decrease of soil pH, with a significant increase of exchangeable Al and a reduction of exchangeable
493 base cations, which limit the plant growth and reduce the microbial biomass. Consequently a
494 reduction of the soil TOC and TN content was observed in the N addition plots.

495 Under the shallow snow cover a lower content of organic matter, microbial biomass and inorganic
496 N forms than the other snow treatments was found. Under the shallow snow cover the N addition
497 caused a decrease of soil pH, which reached values higher than values measured in the N addition
498 plots under moderate snow cover, causing only a slight increase of exchangeable Al, mainly in the
499 subsoil, but no significant effect on exchangeable base cations. Here the N addition caused a great
500 increase of TOC and TN both in the topsoil and subsoil, and of DOC and inorganic N forms in the
501 topsoil.

502 Under the deep snow cover the organic matter and total nitrogen content was slightly greater than
503 under the shallow snow cover, but lower than under the moderate snow cover, revealing how in this
504 zone the soil properties are affected by the significant snow accumulation. Under the deep snow
505 cover the N addition caused a slight decrease of soil pH and consequently the exchangeable Al only
506 slightly increased. The TOC, TN, and inorganic N content slightly increased due to the N addition,
507 while the microbial biomass was unchanged.

508 These results demonstrate how long-term changes in snow properties and excessive N deposition
509 may interact, sometimes with contrasting effects, in affecting alpine soil characteristics, with an
510 important response of soil nutrients. The curvilinear responses often observed here argue that biotic

and soil changes in response to multiple drivers such as climate and N enrichment are produced by the interaction of the factors rather than by linear combinations of these drivers.

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743 **Caption of tables and figures**

744 **Table 1** Mean soil characteristics. Organic matter (OM), Soil colour, C:N ratio (C/N), Water
745 content (WC).

746 **Table 2** TOC, TN concentration in the control plots (DC, MC, SC) and N addition plots (DN, MN,
747 SN) in the topsoil and subsoil. In the column Δ are reported the differences between the N addition
748 and the control plots. * indicate significant differences between means ($p < 0.05$).

749 **Table 3** Mean (\pm standard deviation) N forms (topsoil) in the control and N addition plots under
750 deep (D), moderate (M) and shallow snow covers (S). In brackets % of TN for N forms and % of
751 TOC for C forms. Different letters in the same columns indicate significant differences between
752 means ($p < 0.05$).

753 **Table 4** Results of ANOVA for snow depth and N addition on NH_4^+ (a) and NO_3^- (b)

754 **Table 5** Results of ANOVA for snow depth and N addition on N_{micr} (a) and C_{micr} (b)

755

756 **Figure 1** Alpine mesotopographic gradient at Niwot Ridge (3528 m ASL).

757 **Figure 2** Annual inorganic nitrogen (DIN) wet depositions at Niwot Ridge (data from the NADP
758 program).

759 **Figure 3** Snowfence at Niwot Ridge. In brackets the mean snow depths in the different sectors.
760 Data ($n = 24$) collected at about maximum snow accumulation in 1993 (4/21/1993, gray line) and
761 1994 (4/18/94, black line).

762 **Figure 4** TOC and TN concentration (g/kg) in the topsoil under the different snow cover treatments.
763 Different letters indicate significant differences between means ($p < 0.05$).

764 **Figure 5** TOC and TN concentration (g/kg) in the subsoil under the different snow cover
765 treatments. Different letters indicate significant differences between means ($p < 0.05$).

766 **Figure 6** Total organic carbon (TOC) content (g kg^{-1}) in the topsoil of the control and N addition
767 plots under deep, moderate and shallow snow cover in 2009 and 1993 (pre-snowfence).

768 **Figure 7** Total nitrogen (TN) content (g/kg) in the topsoil of the control and N addition plots under
769 deep, moderate and shallow snow cover in 2009 and 1993 (pre-snowfence).

770 **Figure 8** pH of the topsoil and subsoil under deep, moderate and shallow snow cover, in the
771 control, and in the N addition plots. Different letters indicate significant differences between means
772 ($p < 0.05$).

773 **Figure 9** Exchangeable Al of the topsoil and subsoil under deep, moderate and shallow snow cover,
774 in the control and in the N addition plots. Different letters indicate significant differences between
775 means ($p < 0.05$).

776 **Figure 10** Exchangeable Ca^{2+} of the topsoil and subsoil under deep, moderate and shallow snow
777 cover, in the control and in the N addition plots. Different letters indicate significant differences
778 between means ($p<0.05$).

779 **Figure 11** Exchangeable Mg^{2+} of the topsoil and subsoil under deep, moderate and shallow snow
780 cover, in the control and in the N addition plots. Different letters indicate significant differences
781 between means ($p<0.05$).

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810 **Tables**

811 **Table 1** Mean soil characteristics. Organic matter (OM), Soil colour, C:N ratio (C/N), Water

812 content (WC).

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Site	Horizon	OM	Soil colour	C/N	WC
		%	dry		%
	topsoil				
DC		16.2	10YR2/2	12.9	24.4
DN		17.7	10YR2/2	14.2	33.1
MC		22.9	10YR2/2	13.5	36.7
MN		18.6	10YR2/2	12.9	15.5
SC		14.8	10YR2/2	13.9	20.4
SN		23.0	10YR3/2	13.0	25.6
	subsoil				
DC		9.8	10YR4/3	14.5	15.1
DN		11.2	10YR2/2	13.9	24.9
MC		14.6	10YR2/1	13.5	21.1
MN		10.1	10YR3/3	13.8	17.3
SC		8.1	10YR3/2	13.6	10.3
SN		10.8	10YR3/2	13.8	18.3

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819 **Table 2** TOC, TN concentration in the control plots (DC, MC, SC) and N addition plots (DN, MN, SN) in the topsoil and subsoil. In the column Δ
820 are reported the differences between the N addition and the control plots. * indicate significant differences between means ($p < 0.05$).
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Horizon			DC	DN	Δ	MC	MN	Δ	SC	SN	Δ
topsoil	TOC	gkg^{-1}	94	103	+9	134	108	-26*	86	134	+48*
	TN	gkg^{-1}	7.4	7.3	+0.1	9.9	8.4	-1.5*	6.2	10.2	+4.0*
subsoil	TOC	gkg^{-1}	56.9	64.9	+8.0	84.8	59.4	-25.4*	46.8	62.7	+15.9*
	TN	gkg^{-1}	3.9	4.7	+0.8	6.3	4.3	-2.0*	3.4	4.5	+1.1*

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836 **Table 3** Mean (\pm standard deviation) N forms (topsoil) in the control and N addition plots under deep (D), moderate (M) and shallow snow covers
837 (S). In brackets % of TN for N forms and % of TOC for C forms. Different letters in the same columns indicate significant differences between
838 means ($p < 0.05$).

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		NH_4^+		NO_3^-		DON		DOC		C_{micr}		N_{micr}		C/N_{micr}
		mgkg^{-1}		mgkg^{-1}		mgkg^{-1}		mgkg^{-1}		mgkg^{-1}		mgkg^{-1}		
	D	4.26 \pm 0.91a	(0.06)	2.45 \pm 0.77b	(0.02)	13.05 \pm 1.90c	(0.18)	170.57 \pm 23.17b	(0.18)	1086.74 \pm 148.34b	(1.15)	135.43 \pm 15.73c	(1.84)	8.01 \pm 0.17a
Control	M	4.96 \pm 0.54a	(0.05)	1.82 \pm 0.55ab	(0.02)	24.04 \pm 1.70ab	(0.24)	266.62 \pm 6.19ab	(0.20)	1450.93 \pm 91.93a	(1.09)	167.05 \pm 17.17a	(1.69)	8.72 \pm 0.46bc
	S	2.52 \pm 0.68b	(0.04)	0.87 \pm 0.10a	(0.01)	19.02 \pm 3.81bc	(0.31)	177.63 \pm 36.66b	(0.21)	933.22 \pm 144.97b	(1.08)	102.71 \pm 12.41b	(1.65)	9.07 \pm 0.51c
	D	6.35 \pm 0.54c	(0.09)	2.55 \pm 0.29b	(0.03)	20.62 \pm 2.01b	(0.28)	227.05 \pm 21.52bc	(0.22)	1085.17 \pm 98.61b	(1.05)	133.34 \pm 7.38bc	(1.82)	8.13 \pm 0.44ab
Nadd	M	4.80 \pm 0.95a	(0.06)	3.07 \pm 1.18bc	(0.04)	31.66 \pm 5.21a	(0.38)	338.29 \pm 31.67a	(0.31)	978.12 \pm 94.80b	(0.90)	107.15 \pm 13.31bc	(1.28)	9.17 \pm 0.66c
	S	7.51 \pm 1.15c	(0.07)	3.57 \pm 0.45c	(0.03)	25.43 \pm 9.16ab	(0.25)	292.81 \pm 93.54ac	(0.22)	1102.18 \pm 153.68b	(0.82)	131.79 \pm 14.66bc	(1.29)	8.34 \pm 0.32a

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842 **Table 4** Results of ANOVA for snow depth and N addition on NH₄⁺ (a) and NO₃⁻ (b)

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a)				
Variable	df	MSE	F	P
N addition	1	31.945	46.768	0.000
Snow depth	2	0.387	0.567	0.577
N addition * Snow depth	2	13.346	19.539	0.000
error	12.295			
b)				
Variable	df	MSE	F	P
N addition	1	10.966	25.466	0.000
Snow depth	2	0.179	0.415	0.667
N addition * Snow depth	2	3.409	7.916	0.003
error	7.751			

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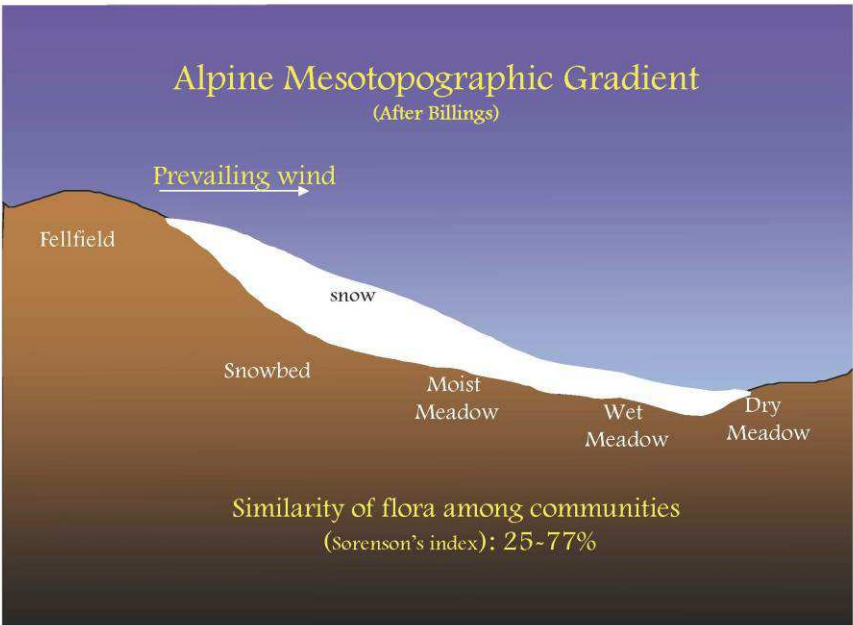
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862 **Table 5** Results of ANOVA for snow depth and N addition on N_{micr} (a) and C_{micr} (b)
863

a)				
Variable	df	MSE	F	P
N addition	1	721.25	3.788	0.067
Snow depth	2	927.100	4.868	0.020
N addition * Snow depth	2	4076.524	21.404	0.000
error	18			
b)				
Variable	df	MSE	F	P
N addition	1	62188.785	3.978	0.061
Snow depth	2	79904.356	5.111	0.017
N addition * Snow depth	2	221003.329	14.137	0.000
error	18			

864 **Figures**

865 **Figure 1** Alpine mesotopographic gradient at Niwot Ridge (3528 m ASL).



897 **Figure 2** Annual inorganic nitrogen (DIN) wet depositions at Niwot Ridge (data from the NADP
898 program).

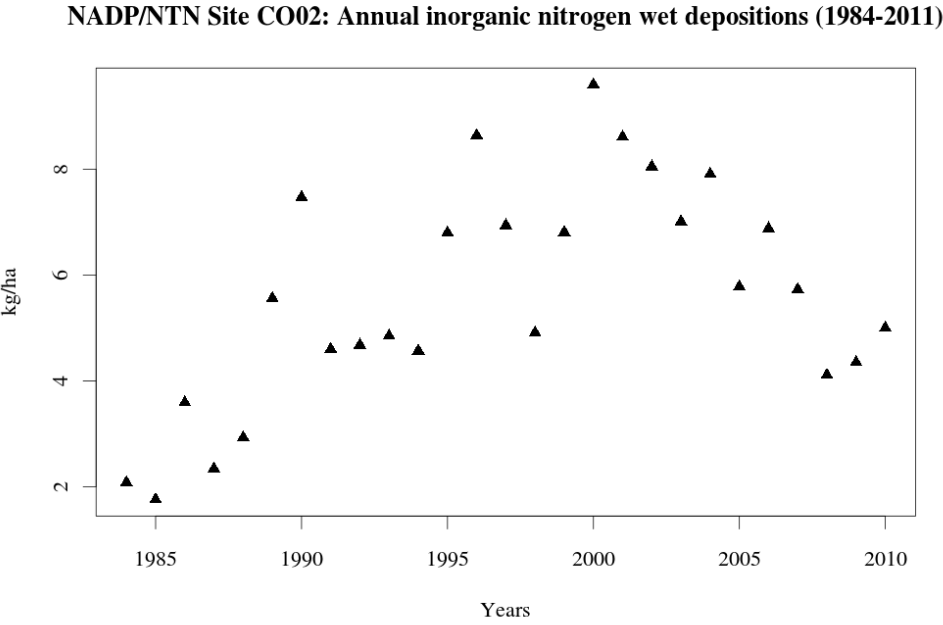
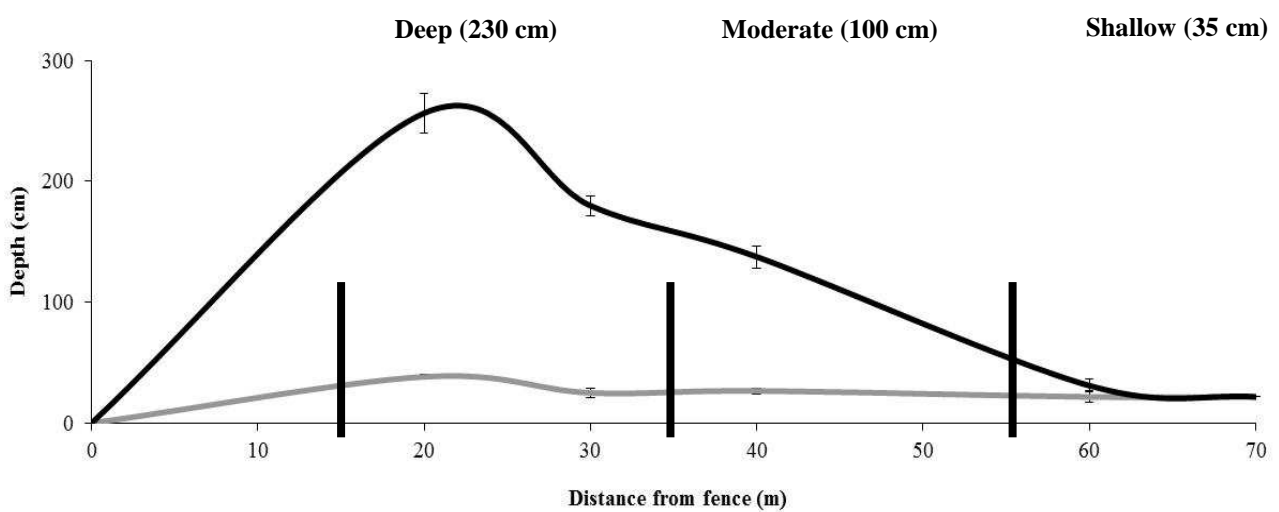


Figure 3 Snow fence at Niwot Ridge. In brackets the mean snow depths in the different sectors. Data (n= 24) collected at about maximum snow accumulation in 1993 (4/21/1993, gray line), prior to the snowfence setup, and 1994 (4/18/94, black line).



965 **Figure 4** TOC and TN concentration (g/kg) in the topsoil under the different snow cover treatments.
 966 Different letters indicate significant differences between means ($p < 0.05$).
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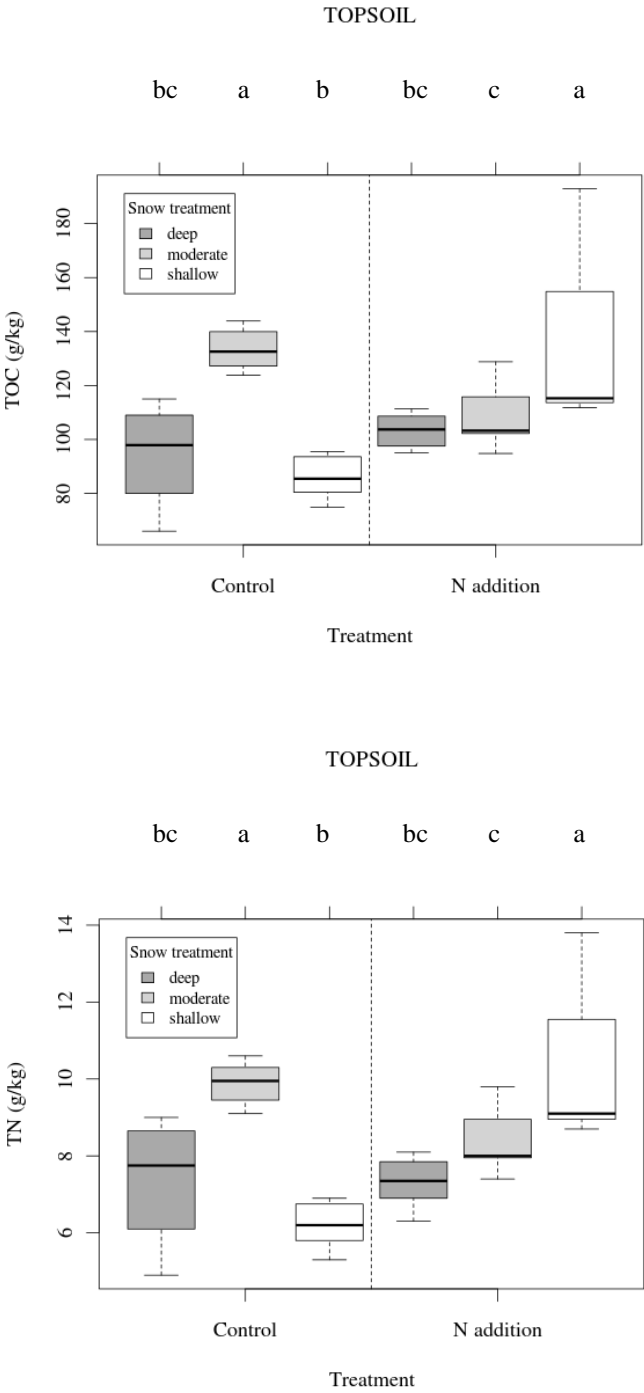


Figure 5 TOC and TN concentration (g/kg) in the subsoil under the different snow cover treatments. Different letters indicate significant differences between means ($p < 0.05$).

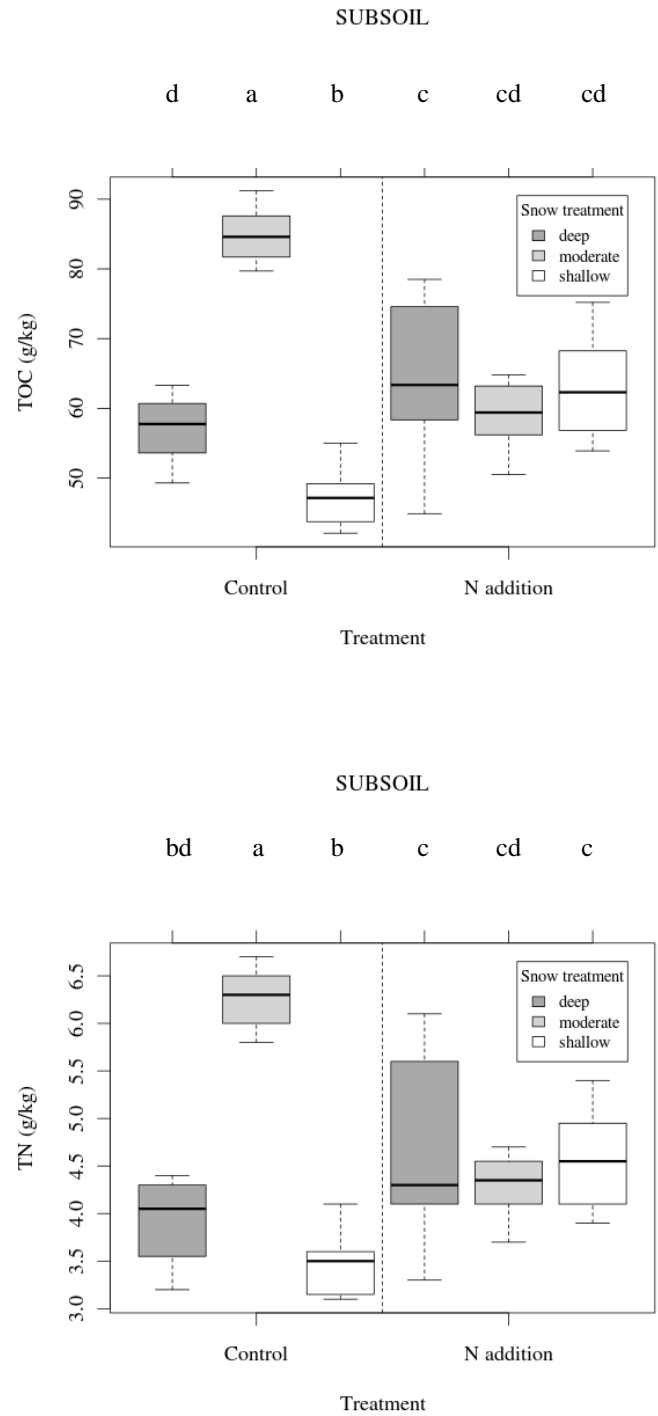


Figure 6 Total organic carbon (TOC) content (gkg^{-1}) in the topsoil of the control and N addition plots under deep, moderate and shallow snow cover in 2009 and 1993 (pre-snowfence).

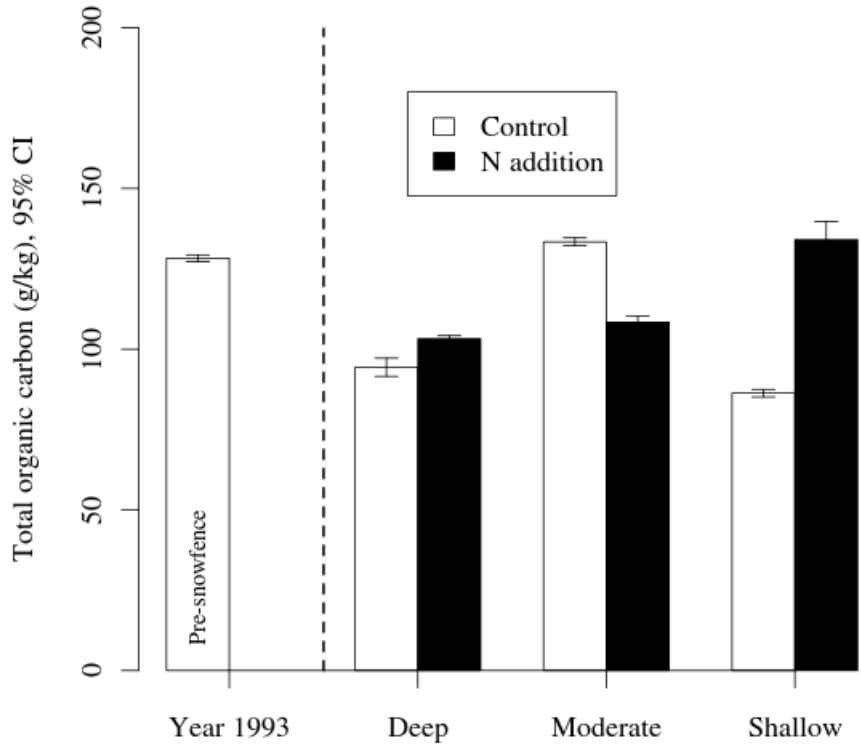


Figure 7 Total nitrogen (TN) content (g/kg) in the topsoil of the control and N addition plots under deep, moderate and shallow snow cover in 2009 and 1993 (pre-snowfence).

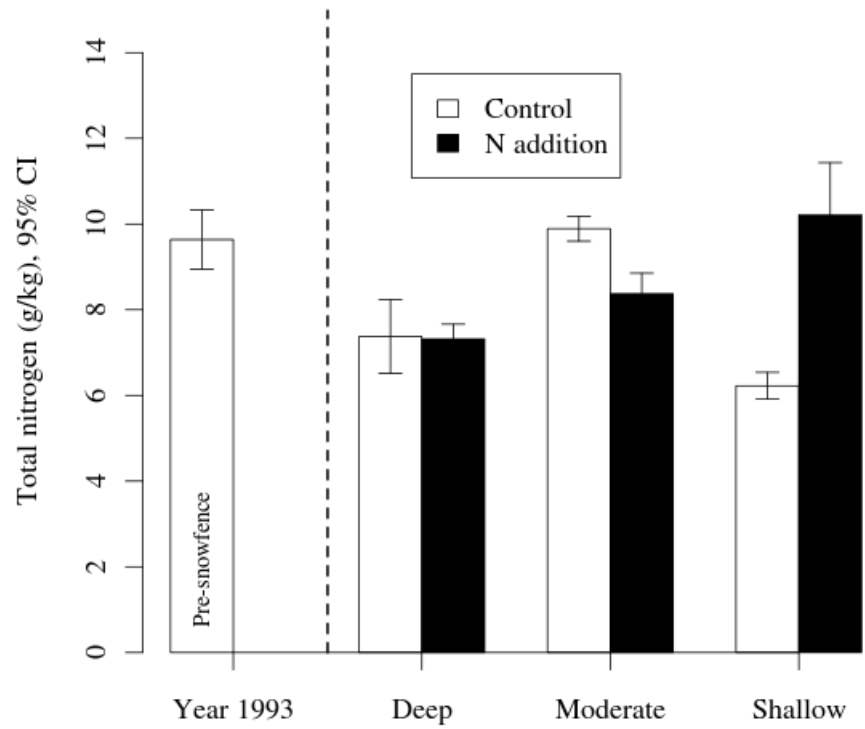


Figure 8 pH of the topsoil and subsoil under deep, moderate and shallow snow cover, in the control, and in the N addition plots. Different letters indicate significant differences between means ($p < 0.05$).

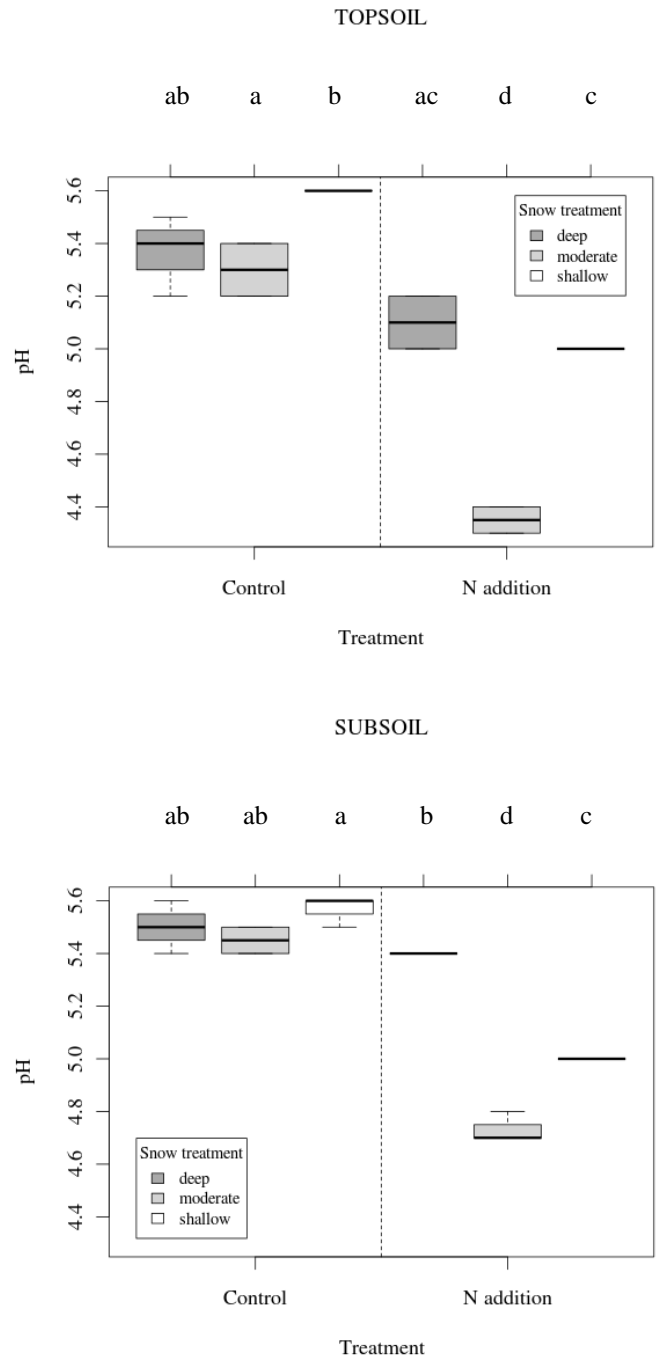


Figure 9 Exchangeable Al of the topsoil and subsoil under deep, moderate and shallow snow cover, in the control and in the N addition plots. Different letters indicate significant differences between means ($p < 0.05$).

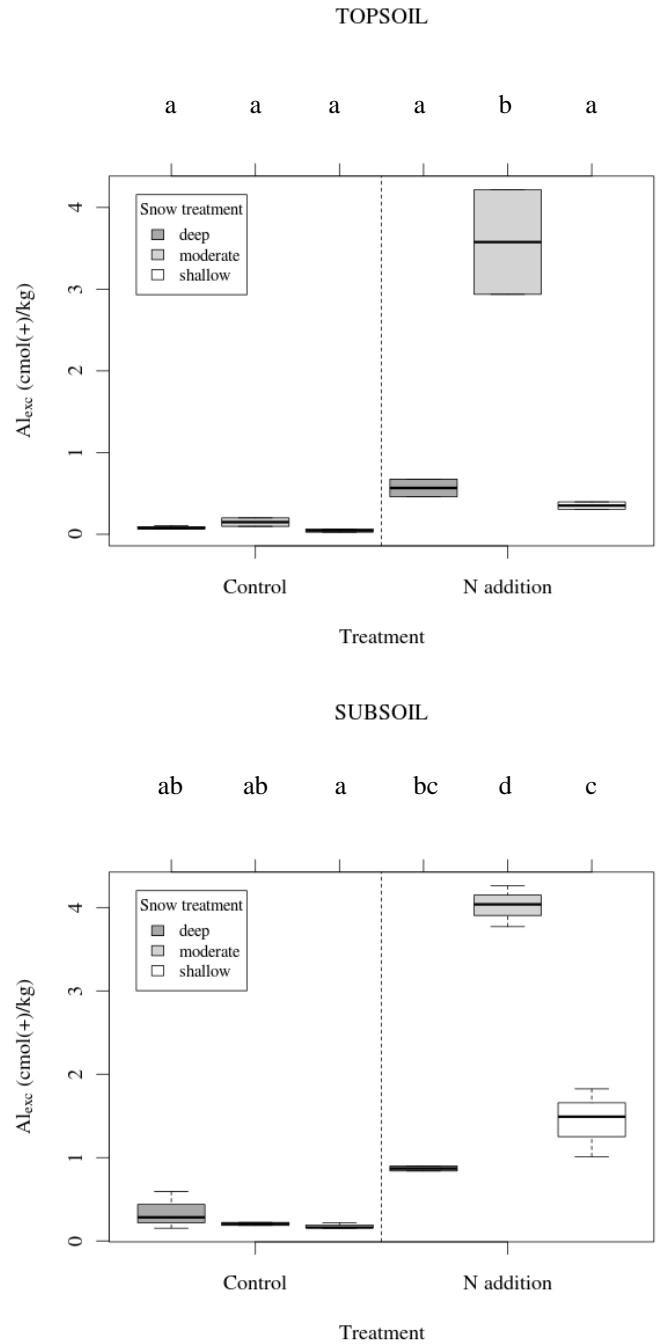


Figure 10 Exchangeable Ca^{2+} of the topsoil and subsoil under deep, moderate and shallow snow cover, in the control and in the N addition plots. Different letters indicate significant differences between means ($p < 0.05$).

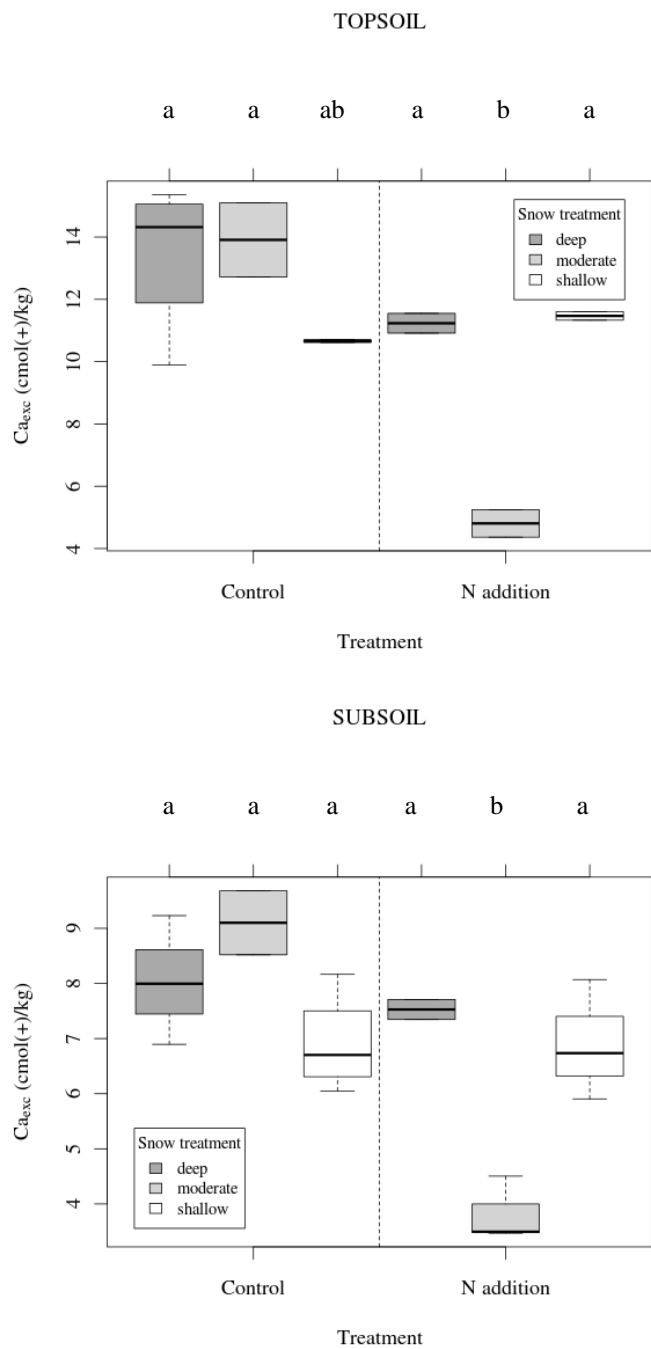
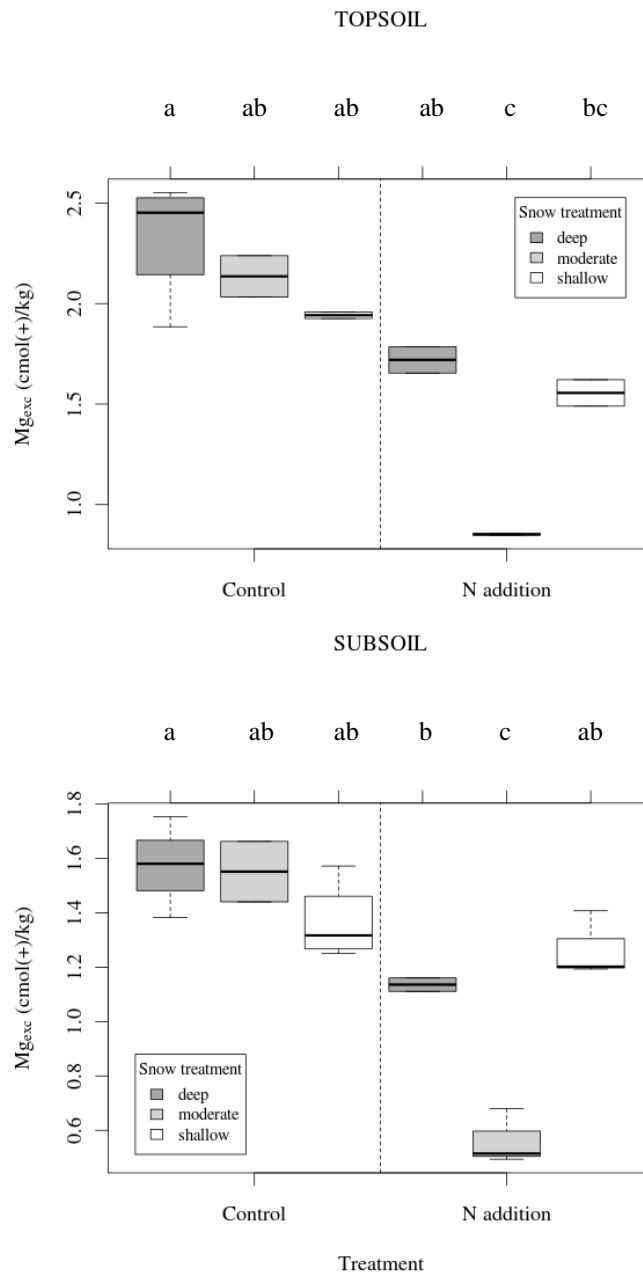


Figure 11 Exchangeable Mg^{2+} of the topsoil and subsoil under deep, moderate and shallow snow cover, in the control and in the N addition plots. Different letters indicate significant differences between means ($p < 0.05$).



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